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Effect of Pesticide on Fish *Labeo Catla* (*Cyprinus Catla*)

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ABSTRACT : Unsafe spraying and improper handling of the chemical pesticides may cause high risk of the health hazards. Aquatic ecosystems are the ultimate sinks for agricultural residues as well industrial pollutants and it has become a global environmental problem in recent days. The environmental condition like temperature, pH and dissolved oxygen play major role to increased pesticide toxicity in the presence of residual molecules. The biological activity of phenthoate, which directly inhibit enzyme, acetyl cholinesterase (Ache) activity in fish, increased protein turn over and enhanced the glycolytic activity. *Labeo Catla* is one of the most preferred edible species in Madhubani district where the present study was carried out. However, its performance in terms of growth is slower compared to other species in the multispecies culture system. The most of synthetic organic pesticides of organophosphates, carbomates and organochlorides are extremely toxic to non-target species of freshwater fauna. Fishes are particularly very sensitive to the water contamination. Hence, pollutants such as insecticides, herbicides may significantly affect some physiological and biochemical processes when they enter into the organs of fishes.

KEYWORDS: Biological, Phenthoate, Multispecies, Organophosphates, Fish and Freshwater.

I. INTRODUCTION

Reservoirs cover only a very small portion of earth's surface. Freshwater Reservoirs are the wealth of a nation, being a source of water for both irrigation and drinking. The surface water is an integral part of natural environment and its quality is very sensitive and critical issue in many countries. Also, with an increased understanding of the importance of drinking water quality to public health and raw water quality to aquatic life, there is a great need to assess surface water quality. Freshwater ecosystems are getting deteriorated because of the developmental activities and highest degradation are caused by humans and their related activities in their immediate catchment areas and drainage basins (Mishra and Garg 2011, Amin et al. 2014). Water quality deterioration in Reservoirs has recently been a matter of great concern due to its negative impacts on social economic and health aspects and has been recognized as a serious problem at local regional and global levels. Due to population growth and industrial development, numerous water bodies have become heavily polluted over past several years. The major problem with the Reservoir is implemented by rainy water or other feeding sources that once contaminated, it is defaulted restore its quality (Dubey 2016). Water quality is the characteristics of water which influence its beneficial use as well as the sustainability of ecosystem. The quality of surface water is governed by the natural and anthropogenic processes including rainfall, erosion, hydrological features, industrial and agricultural activities (Bhat and Pandit 2014). Water is the most precious gift of nature which is essential for sustaining all forms of life, food production and economic development.. Fresh water is essential for sustenance of life human, animal and plant. About 65% of human body is water; 5% of it is to be replaced every day. Water serves as a medium in which the plants and animal of reservoir and characteristics are greatly affected due to discharge of domestic, municipal, industrial and other several factors during the day and night which may be fatal for aquatic life.

These waters contain excess of organic matter, excess of nitrogen and phosphorous, suspended solids, chloride and pathogenic organisms. They also get a lot of garbage, effluent and sewage. These control the nature of vegetation and fauna of the aquatic body. Phosphorous and nitrogen inputs from the domestic wastes, fertilizers accelerate the process of eutrophication of water. Though beneficial in terms of secondary production, eutrophication sometimes promote

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blooms of blue green algae which alter food chain sequences leading to production of commercially less valuable higher tropic organisms. The maintenance of a healthy aquatic ecosystem is dependent on the physico-chemical properties of water and biological activity. Further water condition play a very important role in the production of air breathing fishes. The polluted state of water resources has led to steady decline in aquatic productivity. Therefore, limnological investigation is needed. The maintenance of healthy aquatic ecosystem is dependent on the physico-chemical properties of water. So, the monitoring of the reservoir water is necessary step to mark the trend pattern of pollutants and their effect on living organisms.

Unsafe spraying and improper handling of the chemical pesticides may cause high risk of the health hazards. Aquatic ecosystems are the ultimate sinks for agricultural residues as well industrial pollutants and it has become a global environmental problem in recent days. The environmental condition like temperature, pH and dissolved oxygen play major role to increased pesticide toxicity in the presence of residual molecules. The biological activity of phenthoate, which directly inhibit enzyme, acetyl cholinesterase (Ache) activity in fish, increased protein turn over and enhanced the glycolytic activity. *Labeo Catla* is one of the most preferred edible species in Madhubani district where the present study was carried out. However, its performance in terms of growth is slower compared to other species in the multispecies culture system. The most of synthetic organic pesticides of organophosphates, carbomates and organochlorides are extremely toxic to non-target species of freshwater fauna. Fishes are particularly very sensitive to the water contamination. Hence, pollutants such as insecticides, herbicides may significantly affect some physiological and biochemical processes when they enter into the organs of fishes. The quality of water in reservoirs are determined by physico-chemical and biological parameters. Hence monitoring of these aquatic resources is crucial for sustainable management. More over the insecticides mainly impacts on liver of fish and also decline glycogen content in liver. The mode of action of toxicants and causes for death of poisoned aquatic animal is better understood from biochemical investigations besides mortality studies. Several workers have investigated the impact of various heavy metals on biochemical constituents of various aquatic organisms. Hence, the present study was undertaken to evaluate the toxicity of phenthoate (50% EC), on glycogen and protein levels of liver, kidney, gill, brain, muscle and gut in freshwater Indian major carp *Labeo Catla* (Hamilton).

II. REVIEW OF LITERATURE

Review of selected literature is an essential part of every research process. It helps us to examine and evaluate what has been said earlier on the research subject.

Nikam S.M, et.al (2011) :They have studied the acute toxicity of organophosphate pesticide. Metasystox have been studied on fresh water fish *Nemachellus botia*. Fish became restless and started moving to & fro in water. Fish started coming on the surface of water to inhale air thus started suffocating within 24 hrs. Paralysis of body parts was observed in fishes. The LC50 values and exposure period showed a direct relationship.

Ladipo M. K. (2011): His study reveals the toxicity of Paraquat dichloride on fishes. It gives the information of acute toxicity. The LC50 value based on probit analysis is found to be 60 mg/ L for 96 hrs of exposure to herbicide. There was no mortality of fish in the control experiment throughout the 96 hrs. There was no mortality of fish exposed to 20 mg/L. There was 50% mortality at 60mg/L while at 110 mg/L , 100% mortality was observed. The behaviour of the control fish introduced to different concentrates showed different abnormal behaviour. Abnormal behaviour such as erratic swimming sudden quick movements & restlessness were observed in fish exposed to chemical. At high concentration of mg/L fish became very weak & settled at the bottom.

K.Suneetha (2012): She has studied the effect of endosulfan and fenvalerate on carbohydrate metabolism of the freshwater fish *labeo rohita* (Hamilton). Her study has indicated the liver was vital organ of carbohydrate metabolism & were drastically affected by both endosulfan & fenvalerate. In almost all the tissues of fish brain , gill, kidney, liver, & muscle tested at sublethal & lethal concentrations of both endosulfan & fenvalerate a decrease in glycogen values was noticed during the exposure periods. Among various tissues higher glycogen content was observed in liver this is due to the involvement of liver in glycogen synthesis of utilization. liver is drastically affected.

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Mukesh Kumar Napit (2013): He has studied on the toxicity of pesticides cause diseases behavioral abnormalities, cancer & gene mutations in fishes. The most frequent used pesticides are insecticides, herbicides, & fungicides. They are two types of pesticides natural & synthetic. Also he has explained that in synthetic pesticide organophosphate is most toxic to vertebrate. Pesticide also cause toxic effect on fish as behavioral abnormalities include cancer hematological & biochemical change have been observed.

Suchismita Das (2013): Studied among the wide majority of pesticides ,dichlorvas (2-2, dichlorovinl dimethyl phosphate) commonly used agricultural pesticide. It is presently toxic to organisms like fish, hampers fish health, low metabolism sometimes leading to death. Histopathology study showed effects of liver tissue in Cirrhinusmrigala chronically exposed to dichlorvas showed hepatic lessions in the liver tissue which were characterized by cloudy swelling. Changes in gills are obseved, clumping of cytoplasm & rupture of the cell wall. Dichlorvas toxicity in fish has shown adverse effect as developmental changes, mutagenesis & carcinogenicity.

III. MATERIALS AND METHODS

Healthy juvenile fish *Labeo Catla* measuring with 7.5 ± 1.5 cm in length and 8.5 ± 0.5 g in body weight were obtained from Local fish farm Madhubani district, Bihar, India. The fishes were acclimatized to laboratory condition for two weeks in large plastic pools of 100 L capacity containing well aerated unchlorinated ground water, previously washed with 0.1% KMnO4 solution to free walls from microbial infection and physico-chemical characteristics of water were carried out by standard methods. During acclimatization fishes were fed with fish pellets and rice bran on every day after renewing the water.

Table : 1
Physico-chemical analysis of water

Water quality parameter	Calculated value
Temperature	$30 \pm 3^\circ$
Turbidity	7.6 silica units
pH value at 30°C	7.70
Total hardness as (CaCO ₃)	212 (mg/L)
Total suspended solids (TSS)	6 (mg/L)
Chemical oxygen demand (COD)	Nil
Biological oxygen demand (BOD)	8-10 ppm
Sulphates as (SO ₄)	Trace amount
Phosphates	Trace amount
Dissolved oxygen (DO)	5-6 mg/L

Acute Toxicity Study:

Phenthoate stock solution was prepared by dissolving 1 gm of pesticide in 100 mL of acetone and the required quantity of phenthoate was drawn from the stock solution to maintain the concentration of 1 mg/L in the container. The fish were separated into several groups, each containing 10 individuals (n = 10), pilot experiments were conducted to determine the safe sub lethal (LC0), Lethal (LC100) and Median (LC50) concentrations for acute toxicity estimation range from 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6 and 4.0 mg/L for 12, 24, 36 and 48 hr in the experiment. During the whole process a control group (without toxicant) was maintained with acetone for comparison. The lethal concentration values obtained by the following method given by the committee of aquatic toxicity. The experimental design included three replicates. The fish were not fed on the day before the beginning of the experiment. Each day number of dead fish were counted and removed immediately from the test container. The percentage mortality was calculated and the values were pooled up into probit scale. These values were determined and analyzed by using Finney's probit analysis method. Then, ten fish were exposed to sub lethal concentrations of 1/10th of 48 hr of LC50 value (i.e., 0.21 mg/L) for 1, 4 and 8 days, respectively. A suitable control was also maintained to nullify any other effects that likely to affect the fish.

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Then the fishes were dissected immediately. Fresh wet tissues of vital organs viz., brain, gill, liver, kidney, gut, muscle were isolated and preserved in 5% to 10% formalin. The control fish tissues (n = 10) were also processed in the same method for biochemical estimation of the total proteins and glycogen by the standard recommended protocols.

Table : 2

Estimation of LC0, LC50 and LC100 values of *Labeo Catla* exposed to phenthoate for 12, 24, 36 and, 48 hours

S. No.	Period of study (hr)	LC0 values (mg/L)	LC50 values (mg/L)	LC100 value
1.	12	0.6	3.0	6.0
2.	24	0.52	2.6	5.2
3.	36	0.46	2.3	4.6
4.	48	0.42	2.1	4.2

Total Protein Estimation :

The total protein content of the pesticide exposed tissue samples were estimated according to modified standard method of Lowery et al. The quantity of 5% homogenate of brain, muscle, gill and 2% of kidney, intestine tissue were isolated and precipitated with 5% trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 mins. The precipitate was dissolved in 1 mL of 1 N NaOH solution and 0.2 mL of extract taken into test tube and mixed with 5 mL of alkaline copper solution was added. To this 0.5% mL of 50% folin phenol reagent was added. In the time following 30 mins, the optical density was measured at 540 nm against a blank. The standard graph was plotted by using Lowry method with bovine serum albumin. The values were expressed as mg/g wet weight of the tissue.

Glycogen Estimation :

The glycogen was estimated by the standard method of Kemp et al. 5% homogenate of gill, brain, muscle, gut and 2% homogenate of liver and kidney tissues were prepared in 80% methanol and centrifuged at 3000 rpm for 10 mins. The tissue residue was suspended in 5 mL of trichloroacetic acid (TCA), boiled for 15 mins at 100°C, and then cooled in running water. The solution was made up to 5 mL with TCA to compensate the evaporation and then centrifuged. From this, 2 mL of supernatant was taken into the test tube and 6 mL of concentrated H₂SO₄ was added and the mixture was boiled for 10 mins. The mixture was cooled and the optical density was measured at 520 nm. The standard graph was plotted with D-glucose by using the aforesaid method. The glucose was converted to glycogen by the multiplication factor of 0.98 and is expressed as mg of glycogen/g wet weight of the tissue.

IV. RESULTS AND DISCUSSION

The percentages of dead fishes for different phenthoate doses of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mg/L were determined for 12 hr and 48 hr LC50 was found in fingerling of *Labeo Catla*. With increasing concentration of the pesticide the mortality rate of the fingerlings has gradually increased. Then the lethal (LC100), median (LC50) and safe sub lethal (LC0) levels of the toxicant values were evaluated during 12-48 hr exposure period of experimentation.

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Table : 3
Effect of phenthoate on survival of *Labeo Catla* for 12 hr exposure time

S. No.	Conc. of toxicant (mg/L)	log conc.	No. of exposed	No. of dead	Percent of mortality	Probit mortality
Control	-		10	0	0	-
1	0.5	-0.2218	10	0	0	-
2	1.0	0.0791	10	0	0	-
3	1.5	0.2550	10	1	10	3.72
4	2.0	0.3802	10	3	30	4.48
5	2.5	0.4771	10	4	40	4.60
6	3.0	0.5563	10	6	60	5.25
7	3.5	0.6232	10	6	60	5.34
8	4.0	0.6812	10	8	80	5.84
9	4.5	0.7323	10	8	80	6.10
10	5.0	0.7781	10	10	100	8.09

Table : 4
Effect of phenthoate on survival of *Labeo Catla* for 48 hr in the experimentation

S. No.	Conc. of toxicant (mg/L)	log conc.	No. of exposed	No. of dead	Percent of mortality	Probit mortality
Control	-		10	0	0	-
1	0.40	-0.3767	10	0	0	-
2	0.80	0.0757	10	1	10	3.72
3	1.20	0.1003	10	3	30	4.75
4	1.60	0.2041	10	4	40	4.48
5	2.0	0.3222	10	4	40	4.00
6	2.40	0.4014	10	6	60	5.25
7	2.80	0.4683	10	6	60	5.00
8	3.20	0.5263	10	8	80	5.84
9	3.60	0.5774	10	8	80	6.18
10	4.0	0.6232	10	10	100	8.09

Behavioral Manifestation :

The fish showed normal behavior such as well-coordinated with active movements, static equilibrium, active swimming, normal gill movement, free gulping of air at the surface water, horizontal hanging in the water with natural body color and zero mortality rates were observed in the control group. During the exposure of pesticide for different time periods, at the lower concentration of toxicant i.e., 0.5, 0.7, 0.9 and 1.0 mg/L, the fish showed normal behavioral responses and no mortality was found but at higher concentration i.e., 1.0, 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 mg/L, a number of behavioral changes were observed i.e., fishes frequently coming to the surface of water, tried to jump out of the water, loss of equilibrium, erratic and darting swimming movements, rapid gill movement, vertical hanging, fading of their body colour, increased opercular movements, being lethargic and sluggish, excess mucus secretion all over the body and restlessness. Finally fish stays motionless and open their mouth prior to death in the bottom of the container throughout the test tenures. Lastly at 3.0, 3.5, 4.0, 4.5 and 5.0 mg/L concentration toxicant caused 100% mortality in the experimental group when compared to the control groups for 12, 24, 36 and 48 hr, respectively.

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Biochemical Analysis of Total Protein and Glycogen :

The biochemical parameters viz., total glycogen and total proteins were estimated by standard procedures in six tissues i.e., brain, gill, kidney, liver, muscle and gut of all control fish groups, exposure groups to lethal and sub lethal concentration. 1/10th of Median lethal concentration (0.21 mg/L) was taken as sub lethal concentration and evaluated the rate of depletion in total proteins and total glycogen contents along with the percentage changes in wet tissues of the fish for 1, 3 and 6 days, respectively. No mortality was observed at the exposed sub lethal concentrations of toxicant. From the results, it is clear that there is an appreciable decline in different biochemical constituents of the fish under phenthoate stress.

Fishes are the excellent models for monitoring environmental contamination in aquatic system. In the present study, during acute toxicity test, the fishes exhibited several abnormal behavioral responses such as swimming much more frequently jumping, erratic and darting swimming movements, rapid gill movement, vertical hanging, fading of body colour, increase in opercula movements, lethargic and sluggish, excess mucus secretion all over the body and restlessness. The similar symptoms were observed in mosquito fish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos. These behavioral alterations might have caused from the nervous and respiratory manifestations due to phenthoate exposure. These behavioral alterations can also be considered as symptoms of stress in the treated fish. It is proved that in the present study, the fishes after intoxication with phenthoate were similar to those observed in other studies by peer researchers.

Table : 5
Estimation of total proteins and total glycogen in different tissues of *L. Catla* on sub lethal exposure of phenthoate for 1, 3 and 6 days

Biochemical constituent	Tissues (mg/g)	Control (X ± SD)	Treated (X ± SD)		
			1 day	3 day	6 day
Total Proteins (mg/g)	Brain	113.78	108.66	100.24	92.81
	Gill	110.02	102.99	97.16	89.13
	Kidney	116.60	106.20	9.2	83.53
	Liver	149.57	132.03	119.22	91.05
	Muscle	132.20	112.15	112.15	97.23
	Gut	108.40	11.15	93.14	85.61
Glycogen (mg/g)	Brain	10.88	9.26	7.09	4.16
	Gill	12.99	11.91	7.928	3.898
	Kidney	15.36	13.43	5.718	3.00
	Liver	38.08	30.70	13.83	8.142
	Muscle	22.13	16.94	9.92	5.784
	Gut	10.11	9.88	7.27	3.91

Results are mean (X ± SD) of 5 observations indicates the standard deviation values and are significant at P < 0.005

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Table : 6
Percent change of total proteins and glycogen in *Labeo Catla* exposed to phenthoate for 1, 3 and 6 days

Biochemical constituent	Tissues (mg/g)	Percent change		
		1 day	3 day	6 day
Total proteins	Brain	5.03	11.90	18.43
	Gill	6.38	11.69	18.98
	Kidney	8.92	14.92	28.36
	Liver	11.76	20.29	39.13
	Muscle	8.45	15.17	26.45
	Gut	7.62	14.08	21.02
Glycogen	Brain	14.88	34.83	61.76
	Gill	8.39	38.56	68.56
	Kidney	12.58	62.77	80.47
	Liver	19.38	63.67	78.62
	Muscle	23.48	55.17	7.86
	Gut	2.25	28.09	61.32

The biochemical parameters either increase or decrease in the metabolic rate depending on the site of action. Most of the chemicals pesticides acts as metabolic depressor in the environment and generally causes pressure on biologically active molecules such as proteins, glycogen, carbohydrates and lipids. Proteins are the building blocks of the animal's body, and it is most fundamental biochemical substance to maintain the blood glucose and energy source during the stress period.

Hence, the total protein content of all tissues have been decreased gradually with the percent change 5.03%, 6.38%, 8.92%, 11.76%, 8.45%, and 7.62% in the fish *Labeo Catla* when subjected to toxicant for the period of 1 day. In this study, the maximum depletion was exhibited in liver (149.57 to 132.03 mg/g) followed by kidney (116.6 to 106.20), muscle (132.20 to 112.15), gut (108.4 to 100.15) and brain (113.78 to 108.06 mg/g) and the percent variation was significant over control in all tissues. The proteins reduction might be due to the impaired or low protein synthesis under the toxic stress condition and enhancement of photolytic activity in the organisms.

After 3 days (48 hr) period of exposure the total protein content was progressively depleted with the percent change of 11.90%, 11.69%, 14.92%, 20.29%, 15.17%, and 14.8 % in this six tissues whereas on 6th day protein percent change have been deducted to 18.43%, 18.98%, 28.36%, 39.13%, 26.45%, and 21.02% in the tissues with increase the time period. In both 6 and 6 days of exposure period the protein levels have fallen much in the liver and muscle followed by the kidney, gill, brain and gut. The decline in protein content in the different tissues might be due to degradation of proteins into free amino acids for various metabolic activities, which is supported by Kumar and Gopal.

Glycogen is a sub divisional polysaccharide and major storage of glucose that serve as a form of energy in animals. It plays an important role in the glucose cycle that can be quickly mobilized to meet a sudden need for glucose. The glycogen content in various tissues of *L. Catla* was decreased with increased toxicant concentration in the present experiment. On the 1st day of sub lethal exposure of phenthoate, the maximum glycogen reduction was (23.48%) in muscle followed by brain (14.88%), kidney (12.58%), gill (8.39%) and minimum percentage (2.25%) was in gut of *L. Catla* compared to over control. Depletion of glycogen content in all the tissues might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia. In the fish, skeletal muscle glycogen is very important but it attains only 1-2% wet weight compared to liver. Muscle and liver tissues are a major glycogen parts in animals, which contains full of carbohydrates. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself. In the present study, the glycogen content was found to be significantly reduced in all tissues indicating the excess utilization of carbohydrate to withstand pollution induced toxicosis.

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On 3rd day sub lethal exposure, the glycogen levels rapidly decreased in liver (38.08 to 13.83), kidney (15.36 to 5.718) and muscle (22.13 to 9.920) compared to normal values of the fish where as during 6 days exposure period the higher glycogen depletion found to be 80.47 in kidney and followed by muscle (73.86%), gill (68.56%), brain (61.76%) and gut (61.32%) lower levels was in liver (78.62%). The sudden decline of glycogen content in liver, kidney, gill, brain, muscle and gut tissues is an indication of typical stress response with pesticide concentrations in fish. Venkataramana et al. also reported that the glycogen content was decreased (47.93%) in the muscles of fish to 0.5 ppm Malathion exposure during 12 to 48 hr. The similar results of muscle glycogen could have resulted decline due to anaerobic stress. In the kidney, liver and muscle tissues glycogen content has been reduced progressively in the present study due to the entry of toxic substances into the body and failure of the normal functional mechanism of routine metabolism processes.

V. CONCLUSION

The sub lethal exposure of the phenthoate, an organophosphorus pesticide proved to be moderately toxic to fish *L. Catla*, which effects on the total proteins and glycogen levels of vital organs like brain, gill, liver, kidney, gut and muscle etc. Thus the significant alterations were observed during the exposure for 1, 3 and 6 days, respectively. The decline in both total proteins and glycogen levels could be the possible effects (impact) on the enzyme mediated bio-defense mechanism of fingerlings, which pose serious threats to the aquatic environment as well as human life through food chain.

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